Identifying an animal model to study the extracellular role of the bacterial toxin listeriolysin O

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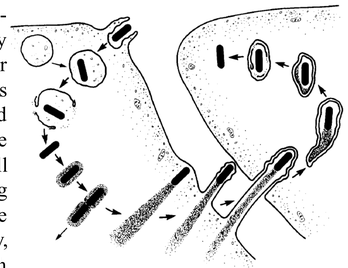
Introduction

Listeriolysin O (LLO) is a cholesterol-dependent cytolysin (CDC) secreted by the foodborne, facultative intracellular pathogen Listeria monocytogenes. This toxin is released in the extracellular and intracellular environments, from where it binds to cholesterol on host cell membranes to form pores, facilitating disease development. Among the numerous members of the CDC family, LLO displays unique properties, such as temperature and pH sensitivity.



Adapted from Tilney and Portnoy. J. Cell. Biol. 1989

At neutral pH and temperatures above 30℃, LLO undergoes denaturation. The kinetic of LLO inactivation at 37°C (PH 7.4) has not been established. Additionally, a recent study proposed that in comparison to human serum, murine serum highly inactivates another CDC family member, pneumolysin (PLY) produced by the bacterial pathogen Streptococcus pneumoniae. If this toxin-inhibitory property of murine serum also applies to LLO, this would imply that the murine model for L. monocytogenes infection is not appropriate to study the extracellular functions of LLO. The first goal of my work is to establish the kinetic curve model for different concentrations of LLO at 37℃ and neutral pH. The second goal is to determine the relative inhibitory activity of sera obtained from various animal species in comparison to human sera in order to establish which animal models would be best to study LLO extracellular activities.

Results

Establish the kinetic curve model for different concentrations of LLO at 37℃ and neutral pH.

LLO activity is known to be temperature and pH sensitive. At neutral pH (pH = 7.4) and at a temperature above 30℃, LLO starts to denature and lose activity. In order to further characterize the hemolytic activity of LLO, I set up 3 different concentrations of LLO: 50 nM, 5 nM and 0.5 nM. The curves of 5 nM and 0.5 nM do not differ greatly.

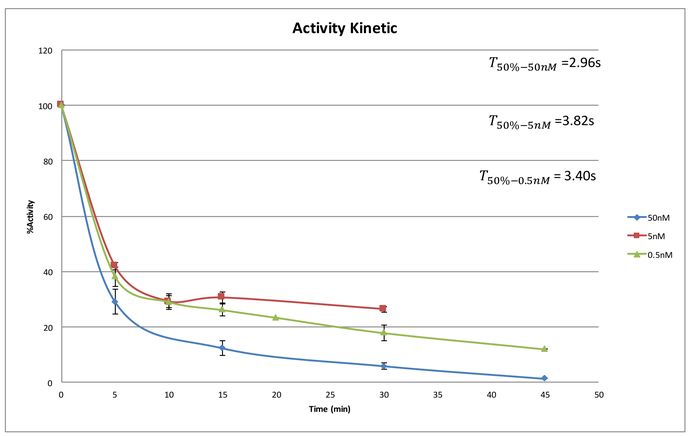


Figure 1. LLO was incubated in PBS at 37℃ for the indicated times and concentrations. At the indicated time points, the tubes were transferred to ice, and the toxin was serially diluted in PBS in a 96 well round bottom plate of 10 ul toxin and 150 ul RBCs (50 nM and 5 nM); 80 ul toxin and 80 ul RBCs (0.5 nM) added on the ice. The plates were then incubated at 37 ℃ for 30 min. Plates were centrifuged at 1500 rmp for 5 min. 100 ul of supernatant was transferred to 96 flat bottom plates for optical density reading at 540 nm. Positive control: RBCs incubated with 10 ul 1% TX-100 used to determine 100% lysis. Negative control: RBCs incubated with 10 ul PBS used to determine 0% lysis

At temperatures above 30℃, LLO irreversibly aggregates. Our data suggest that at higher concentrations, the toxin aggregates at a faster rate than at lower concentrations. According to this reasoning, a higher concentration of toxin will lose activity more quickly than lower one. This hypothesis could be verified by measuring the turbidity over time of the LLO suspensions.

Identifying an animal model to study the role of extracellular listeriolysin O

The first step is verifying Tweten's result of PLY. Based on his paper, exploring the property of PLY.

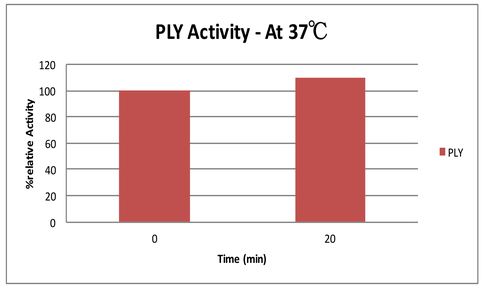


Figure 2. PLY was incubated in PBS at 37℃ for 20 min without serum. After 20 min all tubes were on the ice, and the toxin was serially diluted in PBS or corresponding indicated serum solution in a 96 well round bottom plate of 10 ul txoin and 150 ul RBCs. The plates were then incubated at 37℃ for 30min. Plates were centrifuge at 1500 rmp for 5 min. 100 ul of supernatant was transferred to 96 flat bottom plates for optical density reading at 540 nm. Positive control: RBCs incubated with 10 ul 1% TX-100 used to determine 100% lysis. Negative control: RBCs incubated with 10 ul PBS used to determine 0% lysis

The result shows that toxin PLY is stable after incubating 20 min at 37℃. Next, test the inhibited ability of human serum with PLY.

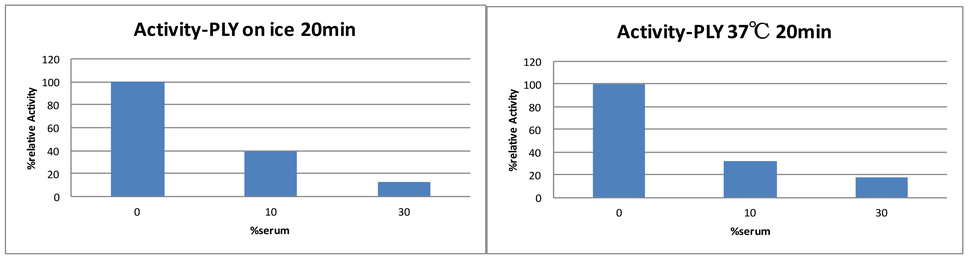


Figure 3 (A) PLY inhibition activity with 10% and 30% human serum under 4℃ 20min. (B) PLY inhibition activity with 10% and 30% human serum under 37℃ 20 min.

Two graphs shows that the inhibited ability of human serum don't have obvious different between two conditions. Human serum can inhibited toxin and 30% serum is more inhibitory than 10% serum. One reason is that antibodies in human serum is able to inhibit toxin. However, Experiments should be repeated with immunoglobulin-depleted serum to evaluate the contribution of lipoproteins. According toTweten, lipoproteins in mouse serum can bind toxin (PLY) to inhibit toxin activity. Thus, it is necessary to test whether human serum has lipoprotein to inhibit toxin by removing the antibodies.

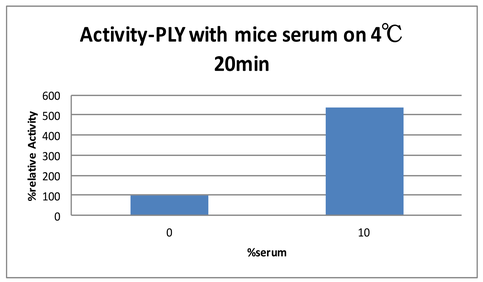


Figure 4. PLY inhibition activity with 10% mice serum

References

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2. Kristin, Wade R., Hotze M. Eileen, Briles E. David, and Tweten K. Rodney. "Mouse, but Not Human, ApoB-100 Lipoprotein Cholesterol Is a Potent Innate Inhibitor of Streptococcus Pneumoniae Pneumolysin." Http://journals.plos.org/. N.p., 4 Sept. 2014. Web. 5 Feb. 2015.

Based upon my preliminary experiments, human serum inhibits PLY to a higher extent than murine serum (figure 3 and 4). The result shows the mice sera don't have inhibited ability for pneumolysin. I could not reproduce the data that were published on pneumolysin either. My hypothesis of this result is mice is not natural host for the pathogen. So it is reasonable that mice cannot inhibit toxin. In addition, I didn't use the same method with Tweten's. The purity of PLY, which I used, may be different with Tweten's. Higher purity toxin has higher toxicity. It is hard to inhibit toxin if the toxicity is much higher. Next, I tested toxin LLO to see whether it have same result with PLY.

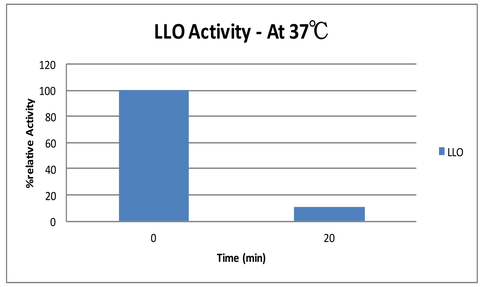

Figure 4. LLO was incubated in PBS at 37℃ or 4℃ for 20 min without serum

The result shows that LLO is temperature sensitive. It will lose lots of activity after incubating 20 min at 37℃. This means toxin PLY is more stable than LLO under 37℃.

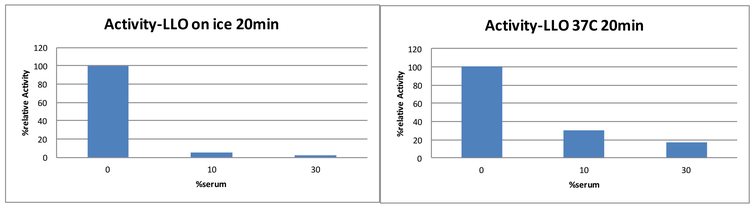

Figure 5 (A) LLO inhibition activity with 10% and 30% human serum under 4℃ 20min. (B) LLO inhibition activity with 10% and 30% human serum under 37℃ 20min.

At the same time, I tested the inhibitory effect of human serum under different incubation temperatures. The complex of human serum and LLO was either incubated on ice for 20 minutes or in a 37℃ water bath for 20 minutes, before measuring LLO hemolytic activity. The results show that human serum is more active when incubated on ice. In addition LLO is temperature sensitive (figure 4). So I cannot incubated serum with toxin LLO at 37℃ to test the inhibited activity, because it is hard to determine what lead to toxin lose activity. Under ICE condition (4℃), I test the mice serum inhibited activity with LLO.

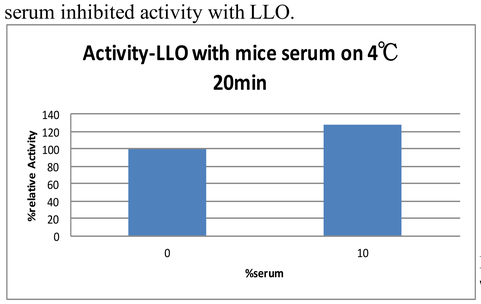

Figure 6. LLO inhibition activity with 10% mice serum

My results shows that mice serum doesn't inhibited toxin LLO, same as results of PLY.

Future research goals

The future research goal is testing other human and murine sera to rule out donor-to-donor variability. I will change my method to test the mice serum inhibition ability with LLO again. Then I will figuring out which animal model is more suitable as a living model to study listeriolysin O. I will test sera of Guinea pig, rabbit and gerbil origin in comparison to human serum. Comparing the sera will allow me to decipher the serum that is similar to human, with respect to listeriolysin O inhibition.

Acknowledgments

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